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Genotoxicity Assessment of Soil Contaminated by Metals/Metalloids Using *Tradescantia pallida*

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Abstract: This study was conducted to study the genotoxicity potential of soil contaminated by the fly ash emitted from coal fired thermal power. The metals/metalloids (Fe, Zn, Pb, Cd, Mo, Cu, Cr, Co and Ni) concentration of contaminated soil (prepared by mixing equal proportion of soil and fly ash, w/w) was estimated with the help of atomic absorption spectrophotometer (AAS) model: AA 7000, SHIMADZU before plantation of *Tradescantia pallida* in pots. Trad-MCN was performed as per standard protocol given by Ma. The image of MCN (micronuclei) was taken with the help of Leica (DM200). It was observed that the presence of metals/metalloids in contaminated soil caused the formation of micronuclei in *Tradescantia pallida* by chromosomal breakage. Present study showed that fly ash contaminated soil can be genotoxic and it can be biomonitored using Trad-MCN bioassay.
Keywords: genotoxicity; metals; bioassay; biomonitoring

INTRODUCTION

The word "Genotoxicity" in genetics is defined as a destructive effect on a cells genetic material (DNA, RNA) disturbing its integrity. A material that has the property of genotoxicity is known as a genotoxins. The genotoxins are mutagens; they can cause mutations which may lead to cancer in test organisms and also humans and includes both radiation and chemical genotoxins. However at the organ level, this indication is common to most of the metals (Punz and Sieghardt, 1993). Many metallic salts are successful mitotic poisons (turbagens) at particular concentration, due to their known affinity for thiol groups and bring about a variety of types of spindle disturbances.

The study on the DNA damage induced by coal dust, fly and bottom ash from coal combustion (from Candiota coalfield in Rio Grande do Sul, Brazil) using the micronucleus test and comet assay *in vitro* have also been performed (Matzenbacher et al, 2017). It was observed that pollutants present in the samples including amount of inorganic elements causes the DNA damage by mechanisms oxidative stress and that preventive measures have to be taken concerning work-related and environmental hazards. The fly ash generated from Plomin coal-fired power plant (Croatia) contaminated the soil of surrounding areas and its impact on the local karstic environment was evaluated by cytotoxic and genotoxic method (Medunić et al, 2016). They proved that soil and ash water extract was cytotoxic to the channel catfish ovary (CCO) cell line.

In the plant systems in vivo, solubility of the metal salt in water is of primary importance. The degree of dissociation and the availability of cations affect the number of aberrations created quantitatively. The viscosity of the plasma membrane may be changed through changes in the ionic environment and formation of chelated complexes, leading to spindle disturbances. Metal attaching to the cell nucleus causes promutagenic injuries including DNA base modifications, inter and intra molecular cross-linkage between DNA and proteins, DNA strand breaks, rearrangements and de-purination. These chemical reactions driving the damages and the resulting mutations are features of an oxidative DNA attack (Kasprzak, 1995). Metal induces formation of ROS (reactive oxygen species) in the DNA surroundings and generates chiefly the promutagenic adduct 8-0xoG (7,8-dihydro-8-oxoguanine) that could miss pair with adenine in the nonexistence of DNA repair, resulting in C to T transversion mutations (Cunningham, 1997). The genotoxic effects of metals depend on the oxidation state of the metal, its concentration and time of its exposure to the test organisms. Generally the effects of pollutants are more distinct at higher concentrations and at longer duration of exposures to pollutants (Bhowmik, 2000). Different plant species respond differently to exposure to the

same metal depending on the number of diploid chromosomes, number of metacentric chromosomes and total length of the diploid complement (Ma et al, 1995). The response varied with the way treatment was given and the plant parts used for exposure (Bhowmik, 2000).

The micronuclei results both from chromosomal fragments and whole chromosomes lagging behind in anaphase, therefore they disclose the clastogenic or aneugenic action of the particular mutagen. Estimation of the numbers of micronuclei in tetrads or MCN frequency of *Tradescantia* was first proposed at the end of the 1970s, in a pioneering study conducted by Ma and groups (Ma et al. 1978). In that study, they used clone 4430 to evaluate the creation of micronuclei in tetrads (Trad-MCN) with stamen hair mutations in cells (Trad-SHM), after exposing the plants to DBE (1,2-dibromoethane). The results obtained showed that the sensitivity of Trad-MCN was approximately 30 times greater than that of Trad-SHM. The greater sensitivity of Trad-MCN in comparison to Trad-SHM has been observed in several other studies also (Gichner and Velemínský, 1999; Minouflet et al. 2005). The Trad-MCN bioassay was developed in 1976 and was used for detecting the gaseous agent, ethylene dibromide and at later stage it was adapted for tests of liquid agents too (Ma 1979, 1990; Ma et al. 1984). In 1984 a confirmation study was carried out in which more than 100 chemicals, common foods and some drugs were tested (Ma et al. 1984). Also, the Trad-MCN assays were used in the monitoring of air pollution (Ma, 1990; Ma et al. 1984), waste water pollutants (Grant, 1998; Grant et al. 1992; Ruiz et al. 1992; Chen and Xiang, 1982) and pollutants of drinking water (Ma et al. 1995). Tradescantia micronuclei bioassay was used for the testing of many well known pollutants that are mutagens and carcinogens (Fang, 1981; Sandhu et al. 1989; Knasmüller et al. 1992).

MATERIALS AND METHODS

Assessments of environmental genotoxicity have been developed using *Tradescantia.* The *Tradescatia* micronuclei tests were used to evaluate for genotoxicity of various environmental pollutants (isík et al. 2011). *Tradescantia* is a genus of perennial herbaceous plants belonging to family Commelinaceae. It originates from the New World and comprises around 500 different species with distribution going from southern Canada to northern Argentina (Watson and Dallwitz, 1992). Micronuclei are structures that results from whole chromosomes or wreckage of chromosomes that, because they do not bind to the spindle fibres, are not included in the nuclei of the daughter cells. However, they remain in the cytoplasm of the interphase cells, where they are observed as small granules resembling the nucleus, measuring 1/10 of the size of the nucleus.

The plants were planted in cemented pots containing contaminated soil (prepared by mixing equal proportion of soil and fly ash, w/w) in the month of August 2016. Pots were kept in the open air, so as to avoid shade, and watered twice a week. Samples were closely inspected to keep them healthy. No fertilizer and pesticides were applied to or around the plants during the experiment so as to avoid their effect. metals/metalloids (Fe, Zn, Pb, Cd, Mo, Cu, Cr, Co and Ni) estimation of contaminated soil was performed with the help of atomic absorption spectrophotometer (AAS) model: AA 7000, SHIMADZU.

Tradescantia-micronucleus (Trad-MCN) bioassay was performed using the protocols established by Ma (1981). Young inflorescences were collected from the flowering plants in the month of November 2016 to February 2017.

RESULTS

In this study an effort has been made for assessing the genotoxicity of metals/metalloids of the soil contaminated by the fly ash (generated from coal fired thermal power plants) using *Tradescantia* micronucleus bioassay (Trad-MCN bioassay). The metals/metalloids concentration of contaminated soil is shown in table 1. It is evident from the table that iron (Fe) was present in maximum concentration and cadmium (Cd) was present in minimum concentration, whereas lead (Pb) and copper (Cu) were not detected in the contaminated soil. It is also clear from the given table that fly ash is an important source of metals/metalloids.

The metals/metalloids concentration present in different plants parts are shown in table 1. The table shows that different plant parts have different accumulating capability and also even for the same metal. Mo, Cu and Cr were not detected in root, stem and leaf; Cd was absent from stem and leaf and Pb from leaf of *Tradescantia pallida* although these metals were present in the contaminated soil. It is also clear that *Tradescantia* plants accumulated metals/metalloids in their body parts and were able to form micronuclei in the *Tradescantia pallida* (figure 1).

Table 1: Metals/metalloids concentration (µg g^{-1} dry wt. of plant parts) present in different plants

Metals/metalloids	Soil	Root	Stem	Leaf
Fe	33.02	19.819	3.517	17.297
Zn	1.402	3.822	1.142	1.990
Pb	*n.d.*	0.310	0.354	*n.d.*
Cd	0.082	0.029	*n.d.*	*n.d.*
Mo	4.49	*n.d.*	*n.d.*	*n.d.*
Cu	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Cr	0.213	*n.d.*	*n.d.*	*n.d.*
Co	0.176	0.115	0.147	0.096
Ni	0.127	0.255	0.310	0.421

n.d. not detected

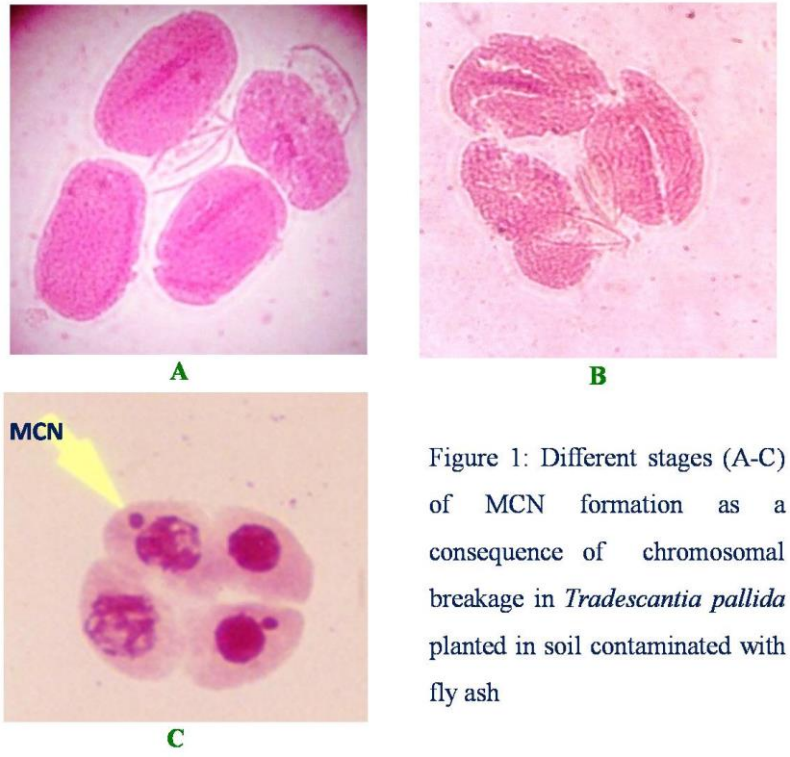

Figure 1: Different stages (A-C) of MCN formation as a consequence of chromosomal breakage in *Tradescantia pallida* planted in soil contaminated with fly ash

DISCUSSION

Plant bioassay developed using plants and clones of the genus *Tradescantia* is considered to be valuable tools for the assessing genotoxic effects of environmental contaminants. The micronucleus test on tetrads of *Tradescantia* (Trad-MCN) is currently the most widely used plant bioassay for detecting genotoxins in the environment (isík et al. 2011). The present study shows that coal fired plants are important source of metals/metalloids pollution coming out of fly ash. The study clearly indicates that metals/metalloids present in the soil was genotoxic in nature and capable to break the chromosomes leading to chromosomal aberrations and formation of micronuclei.

The genotoxicity of metal compounds in the *Tradescantia* have been conducted by determining the initiation of micronuclei (MCN). The present studies have shown that plant bioassays, in particular the Trad-MCN are valuable techniques for the revealing of genotoxic compounds in the environment. This assay is very sensitive towards compounds which cause negative or sensible effects in other widely used systems. For example, metal cannot be detected by bacterial mutagenicity assays, and is also not sensitive towards ionizing radiation. Furthermore, it is important to note that, due to the intensity of most mutagenicity assays, concentration process are required which may lead to loss of active compounds from the substance used for test (Ma, 1981; Ohe et al. 2004) and it can be easily avoided in Trad- MCN experiments. Another reason which favours the use of Trad-MCN assays, concerns the fact that more than 120 individual compounds and about 100 complex environmental mixtures have been already tested (Ma et al. 1984, 2005) and therefore the sensitivity of this assay to different environmental pollution is well known. On the basis of the presently available data, it can be said that

the Trad-MCN bioassay is complementarily to other current methods for the assessment of environmental genotoxins.

This bioassay is based on the revealing of micronuclei which refer to clastogenic (Chromosome breaking) and anuegenic effects in meiotic pollen tetrad cells. The micronuceli frequencies are scored in buds, which contain early tetrads and are highly synchronised. Therefore it can be concluded that the studies demonstrate that *Tradescantia* plants, particularly the Trad-MCN assays, provide a very sensitive, simply manipulated system for the studying genotoxicity, especially under the in situ conditions. Even though mutagenesis in plants and the risk of malignancies in human cannot be equated, Trad-MCN can be very useful as an alternative for assessing human risk.

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